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his-Linked Hydrogen Sulfide Locus of *Salmonella typhimurium* and Its Expression in *Escherichia coli*

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A *his*-linked H_2S locus of *Salmonella typhimurium* has been further defined by direct isolation of H_2S mutants. Expression of this locus in *Escherichia coli* has been demonstrated.

Salmonella typhimurium produces hydrogen sulfide from thiosulfate, whereas *Escherichia coli* does not. By analysis of extended *his* deletions, we previously found that a site affecting H_2S production is located in the region of the *S. typhimurium* chromosome adjacent to the operator end of the *his* operon (9). We also found that an F-*his* plasmid of *Salmonella* origin carried the wild-type allele of the *his*-linked locus (9). We report here the isolation of strains of *S. typhimurium* mutant in H_2S production (*phs*), two of which have mutations closely linked to the *his* operon. Evidence for the expression of the *his*-linked *phs* locus in *E. coli* is also presented. A preliminary report of this work has appeared (M. J. Voll, L. A. Cohen, and J. J. Germida, Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, H68, p. 107).

To test for H_2S production, cultures or clones were stabbed into tubes of peptone iron agar (Difco) or plated on Kligler iron agar (Difco) (*S. typhimurium* strains only) as previously described (9). H_2S substrate specificity was tested by stabbing cultures into tubes of Vogel and Bonner (7) minimal agar supplemented with ferrous chloride and either sodium thiosulfate pentahydrate or sodium sulfite, as described by Lautrop et al. (2). Uninoculated medium controls were included in the assays. F-*his* strains were always cultured in minimal medium lacking histidine before assay for H_2S production.

Conjugations were performed by cross-streaking diluted cultures of donor and recipient strains on selective agar plates. F-ductants were purified on the selective medium before testing for H_2S production. Transductions were performed using P22 *int4* phage as previously described (9).

Cultures of *S. typhimurium hisD477* were treated with UV light or diethyl sulfate by standard mutagenization procedures, subcultured in nutrient broth, and plated to single colonies on Kligler iron agar. About 1,500 to 5,000 colonies were plated in each experiment. Rare pale clones were purified on Kligler iron agar and then tested for H_2S production in peptone iron stabs. Isolates which gave negative reactions in the stab test were assayed for substrate specificity and for linkage of the *phs* mutation to *his* by transductional analysis (Table 1). Nine *phs* mutants were obtained in three selection experiments. Two of the mutants, CP22 and CP23, had *phs* mutations which showed about 30% linkage to *hisD* (second gene in the *his* operon). These two mutants were obtained in the same selection experiment and could be identical. The other mutants did not show close linkage to the *his* operon, although loose linkage cannot be ruled out by the data. We were unable to investigate the molecular nature of the mutation(s) in CP22 and CP23 by reversion analysis, since medium selective for H_2S-producing cells is not available.

None of the mutants produced H_2S from thiosulfate. The parent strain and some of the mutant strains showed a delayed production of H_2S with sodium sulfite as the substrate. On this basis the mutants could be divided into three classes: sulfite reducing, with *phs* mutations linked to *his*; sulfite reducing, with *phs* mutations unlinked to *his*; and sulfite nonreducing, with *phs* mutations unlinked to *his*. The ability of the mutants to reduce sulfite correlated with their ability to reduce nitrate (G. W. Chang, personal communication). The mutations unlinked to *his* have not been mapped.

Two F-*his* plasmids, FS400 and FS401, which carry the *his-gnd* region of *S. typhimurium* have been isolated (8). These plasmids have been maintained in our laboratory in *his* operon deletion strains of *E. coli*. We reported that FS401 carries the wild-type *his*-linked *phs* locus since it restores H_2S production to *phs-his* deletion strains of *S. typhimurium* (9). FS401 also restores H_2S production to strains CP22 and CP23. We reported that FS400 does not carry a wild-type *phs* locus based on F-duction tests using strain SB2063 (FS400/*E. coli* SB2201) (9). Sub-

TABLE 1. *phs mutants of S. typhimurium hisD477*

Isolation[a]	*phs* mutants		H_2S production		Cotransduction of *phs* with *hisD*[b]	
	Strain	Mutation	Thiosulfate	Sulfite	Phs$^+$ His$^+$/His$^+$[c]	%
	hisD477	*phs*$^+$	+	d+[d]		
Expt 1 (DES)	CP22	*phs-1*	−	d+	13/40 13/36	34
	CP23	*phs-3*	−	d+	13/50 35/114	29
	CP24	*phs-4*	−	d+	0/40	<3
	CP25	*phs-6*	−	−	0/50	<2
	CP29	*phs-10*	−	d+	0/31	<4
Expt 2 (UV)	CP27	*phs-8*	−	−	0/50	<2
	CP28	*phs-9*	−	−	0/50	<2
Expt 3 (DES)	CP30	*phs-11*	−	−	0/45	<3
	CP31	*phs-12*	−	d+	0/59	<2

[a] Mutagen used is given in parentheses. DES, Diethyl sulfate.
[b] Strains were transduced to His$^+$ with phage grown on the wild-type strain, CP2. His$^+$ transductants were selected on minimal agar and tested for color reaction on Kligler iron agar plates.
[c] Number of Phs$^+$ His$^+$ transductants/total number of His$^+$ transductants.
[d] d+, Delayed position reaction.

sequently, we have found that the FS400 plasmid carried in *E. coli* strain SB1542 (F-*his*/*E. coli* SB1541) does confer ability to produce H_2S to *S. typhimurium phs-his* deletion strains. We have redesignated the plasmid in strain SB1541 as FS417. Strain SB2063 was constructed by crossing SB1542 with SB2201. It thus appears that FS400 originally carried the wild-type *phs* locus but subsequently lost it through deletion or mutation.

A number of *E. coli his* mutant strains carrying FS401 or FS417 have been tested for H_2S production (Table 2). All the carrier strains contain extended *his* operon deletions except *E. coli hisB463*, which carries a mutation in the *hisB* gene and contains no other known mutation (1, 6). Of the strains tested, two produced H_2S from thiosulfate, XX54 and XX58, carrying FS417 and FS401, respectively. In both strains the carrier strain is *hisB463*. Five His$^+$ colony isolates of XX58 were cultured in nutrient broth supplemented with 20 μg of acridine orange per ml to induce plasmid curing. One His$^-$ colony from each isolate was tested by stabbing into peptone iron agar, and all were found to be H_2S negative. This indicates that in XX58 the Phs$^+$ phenotype is episomally determined.

In a final experiment, the FS401 episome was freshly introduced by conjugation into *E. coli hisB463*, using SB2887 as the plasmid donor and selecting F-ductants on minimal agar. Eight purified F-ductant clones were tested for H_2S reaction in peptone iron agar stabs. Only three of the isolates gave a typical H_2S-positive reaction at 1 to 2 days of incubation. Two gave negative reactions and the other three gave incomplete blackening of the stab growth. A rapid loss of the episome from the majority of clones during growth in the stabs could account for these results.

TABLE 2. *H_2S production in strains of E. coli carrying S. typhimurium F-his plasmids*

Strain	Genotype	Phs phenotype[a]	H_2S production	
			Thiosulfate	Sulfite
SB1541	*hisO-E3157 thr-4 leu-8 ara proA2 lacY1 gal-2 rpsL*[c]	−	ND[b]	ND
hisB463	*hisB463*	−	−	−
RW84	*hisO-E eda edd rpsL* λ^r	−	−	−
SB1844	*hisO-E750 met-77 galK2 ara-14 xyl-5 mtl-1 malA1 rpsL*	−	ND	ND
TA2043	*hisO-E6607 rpsL*	−	ND	ND
SB1542	FS417/SB1541	−	ND	ND
XX54	FS417/*hisB463*	+	d+[d]	−
SB2887	FS401/SB1541	−	ND	ND
XX58	FS401/*hisB463*	+	+	−
XX28	FS401/RW84	−	−	−
XX67	FS401/SB1844	−	ND	ND
	FS401/TA2043	−[e]	ND	ND

[a] Determined by peptone iron agar stabs.
[b] ND, Not determined.
[c] Formerly *strA*^{r}.
[d] d+, Delayed positive reaction.
[e] Eight purified His$^+$ clones from the cross SB2887 × TA2043 were tested and all were negative.

Our results showing that the *Salmonella* F-*his* plasmids can induce H_2S production in an *E. coli hisB* mutant strain suggest that the inability of *E. coli* to reduce thiosulfate to H_2S is due to a deficiency in the *his*-linked *phs* locus. Introduction of the *Salmonella his*-linked locus into

E. coli strains having extended *his* deletions did not lead to H_2S production. These strains may be deleted for some other locus required for H_2S production.

Relevant to the finding that production of H_2S can be episomally induced in *E. coli* are reports of naturally occurring H_2S-producing *E. coli* strains in which H_2S production is attributed to plasmids (3–5).

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